

A gene for pachyonychia congenita is closely linked to the keratin gene cluster on 17q12-q21

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Abstract

Pachyonychia congenita (PC) is a group of hereditary syndromes which have in common a hypertrophic dystrophy of the distal nail, and are associated with a variety of additional features, notably various dyskeratoses of skin and mucous membranes. The pathology is unknown but the array of clinical features suggests the possibility of a keratin abnormality. In the present report we describe linkage analyses in a large PC pedigree of the Jackson-Lawler type, a subtype which is characterised by multiple epidermal cysts, hair abnormalities, and natal teeth. The disease locus in this family was found to be tightly linked to markers mapping within, or very close to, the keratin type I cluster at 17q12-q21; maximum lod scores for linkage of the disease to a KRT10 polymorphism and to D17S800, a marker known to be very tightly linked to KRT10, were respectively +4.51 and +7.73, both at $\theta=0.00$. Although always likely, our findings provide strong evidence of a keratin gene anomaly underlying an inherited disorder affecting epidermis, nail, hair, and mucosa. These findings permit testing to see if pachyonychia congenita shows any locus heterogeneity and suggest specific candidate keratin genes for mutation searching studies. In addition, they suggest a role for keratins in the phenomenon of natal dentition.

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Jackson-Lawler type PC patients often have recurrent flexural infections resembling hidradenitis suppurativa.³⁻⁵ In this type also, natal teeth^{4,6} and peculiarly straight, bushy eyebrow hair are regular features. A third clear group have pachyonychia, macular pigmentation, and amyloid deposition.^{7,8} Other types of PC which have been suggested¹ are based on additional clinical features: corneal lesions and cataract, angular cheilosis, chronic candidiasis,⁹ hoarseness or other laryngeal lesions, alopecia, and mental retardation. There may be variants with onset later in life.¹⁰ In a total of about 200 cases, all these variants are relatively few and the variations minor, so they may not represent distinct subtypes. The pathogenesis of the pachyonychias is unknown, but most if not all of the clinical features are referable to disordered keratinisation. In support of this, ultrastructural histology in Jadassohn-Lewandowsky PC shows marked increases of tonofilaments in the peripheral cytoplasm of keratinocytes from the basal layer upwards.^{11,12} The blistering which occurs in some cases clinically resembles that in epidermolysis bullosa simplex (EBS), being worse in warm weather, and improving with age. EBS is now known to be the result of mutations in genes for the keratins of the basal layer of the epidermis.¹³⁻¹⁵ Mutations in genes for suprabasal keratins cause epidermolytic hyperkeratosis¹⁶⁻¹⁸ and epidermolytic palmoplantar keratoderma.^{19,20} Recently, familial keratosis palmaris et plantaris has also been shown to be tightly linked to the keratin type I gene cluster on 17q.²¹

Keratin genes are clearly also candidate disease genes for each of the pachyonychia congenita syndromes. They are expressed in set pairs, each type I, acidic, keratin being coexpressed with one type II, basic, keratin.²² The pair expressed varies with tissue and circumstances but their genes are almost all clustered on two sites on chromosomes 12q (mostly type II genes) and 17q (type I genes).²² The existence in Glasgow of a very large pedigree of the Jackson-Lawler type provided us with an opportunity to test the hypothesis that PC is the result of keratin defects by investigating linkage of the disease to the keratin gene clusters.

Materials and methods

PATIENTS

Twenty-five affected members of a single family were identified from case records in three Glasgow hospitals, and by tracing of relatives. Cases were ascertained by two clinicians (LK, CM) on the basis of clinical examination (23 cases)

Pachyonychia congenita (PC) is a group of hereditary syndromes which have in common a hypertrophic dystrophy of the distal nail. Numerous classifications based on clinical features have been proposed,¹ but it is uncertain how many genetically distinct diseases exist. Three clinical types based on large pedigrees of autosomal dominant disease are well defined. The major group is the Jadassohn-Lewandowsky type, in which pachyonychia is accompanied by palmoplantar hyperkeratosis, blistering, and hyperhidrosis, with follicular keratoses and oral leukokeratosis. A rarer syndrome was first defined by Jackson and Lawler² in which affected persons also have multiple epidermal cysts (steatocystoma multiplex), but do not have oral leukokeratoses. The cysts are simple epidermal cysts, perhaps arising from vellus hair follicles, but in some reports are true steatocysts with sebaceous glands attached.³

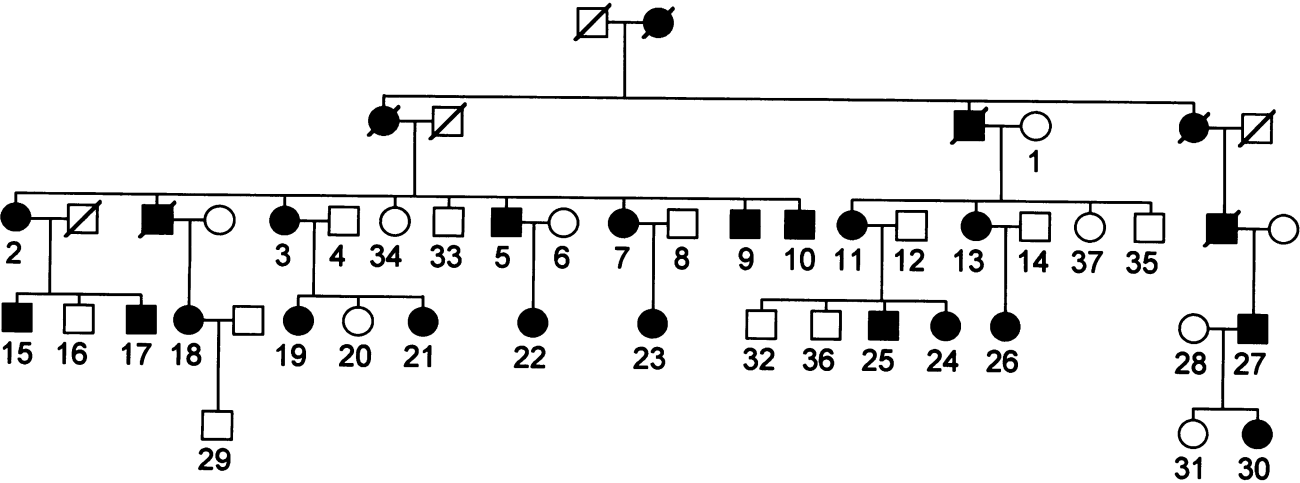


Figure 1 *Pachyonychia congenita* pedigree used for linkage analysis. Numbers of persons in pedigree identify the origin of DNA samples analysed in the corresponding lane numbers in figs 2 and 3.

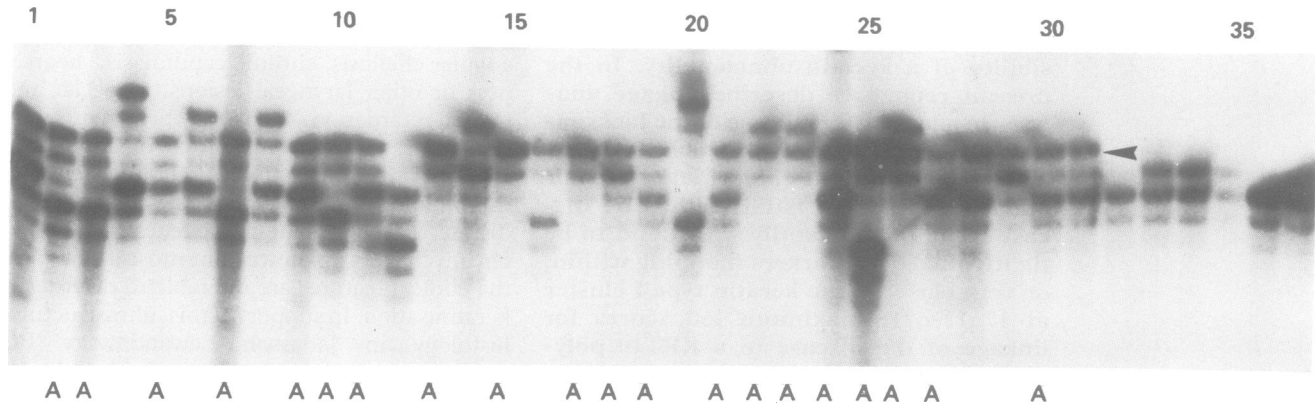


Figure 2 Typing results for microsatellite marker D17S800. Arrow marks the position of the allele segregating with disease. A = affected persons. Lanes 1–37 represent results from typing individual members of the pedigree as numbered in fig 1.

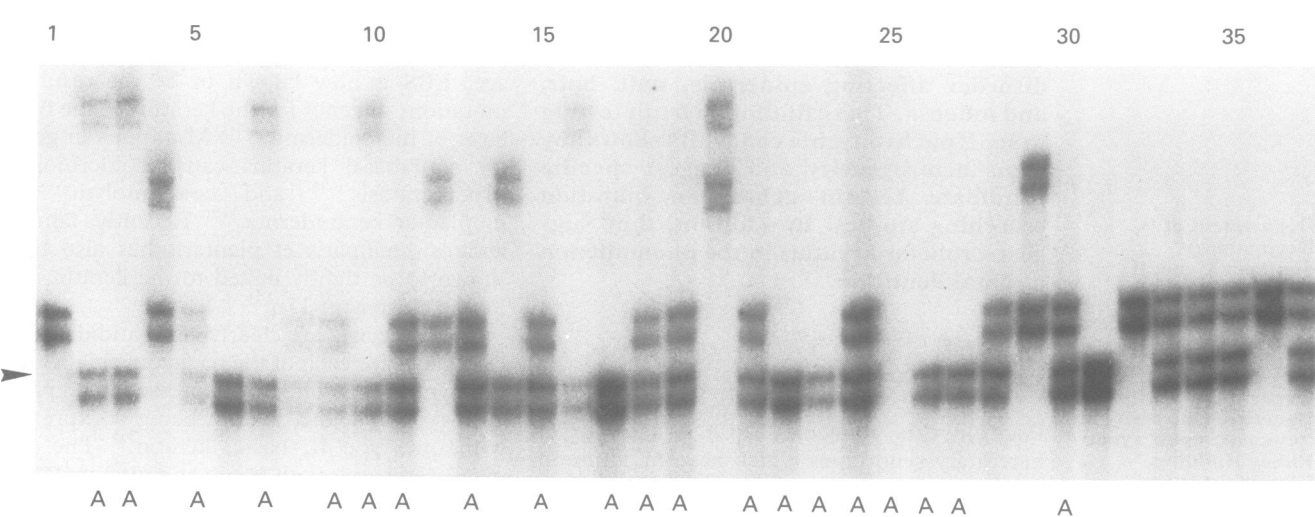


Figure 3 Typing results for a length polymorphism in the *KRT10* gene. The individual alleles for this polymorphism each show two strong closely migrating bands, and four different alleles are apparent in this family. Of these, the smallest allele (identified by an arrow) is the one which segregates with the disease in this family. Lanes 1–37 represent results from typing individual members of the pedigree as numbered in fig 1; the apparent absence of alleles in lane 25 was a result of amplification failure.

Pairwise lod scores for pachyonychia congenita and 17q markers

Marker locus	Lod score for recombination fraction						
	0·00	0·01	0·05	0·10	0·20	0·30	0·40
D17S800	7·73	7·60	7·09	6·43	5·01	3·44	1·69
KRT10	4·51	4·44	4·13	3·74	2·91	2·02	1·05

or telephone interview (2 cases). Blood samples were obtained from 20 affected persons and 17 other members, including 10 unaffected members with an affected parent, as shown in fig 1. Penetrance was complete, although the severity of disease varied (Kunkeler and Munro, in preparation).

DNA TYPING
Microsatellite marker D17S800 and a marker detecting keratin 10 length polymorphism have

previously been described and typed using standard methods as described.^{21,23}

LINKAGE ANALYSIS

Linkage analysis was performed using the data management package LINKSYS²⁴ in conjunction with the computer program LINKAGE version 5.1.²⁵

Results

As described in the Introduction, various findings have suggested that pachyonychia congenita may result from a keratin abnormality. As a first step in testing this hypothesis DNA samples from available members of a large pachyonychia congenita pedigree of the Jackson-Lawler type (fig 1) were typed for polymorphisms known to map in the immediate vicinity of keratin genes. A microsatellite marker D17S800, which is known to be very closely linked to the acidic KRT10 gene,²¹ showed an allele which had consistent segregation with the disease (fig 2). The resulting lod scores were significantly high, with a maximum of +7.73 at $\theta=0.00$ (see table). To verify this result we followed up by typing family members with a marker which identifies extensive length polymorphism within the coding sequence of the KRT10 gene.²³ Again, as shown in fig 3, one allele was consistently found to segregate with the disease, and provided significantly positive lod scores, with a maximum of +4.51 at $\theta=0.00$ (table).

Discussion

The D17S800 marker is known to be very tightly linked to a length polymorphism described by Korge *et al*²³ in the C terminal V2 subdomain of the KRT10 gene product; one recent report showed no recombination between these two markers in at least 25 informative meioses and cited a personal communication which also testified to the absence of recombination between these markers.²¹ Our results confirm the close linkage of these two markers, neither of which show recombination with the disease allele in the large PC pedigree which we have investigated. The KRT10 gene is known to map within a closely clustered group of acidic type I keratin genes, including KRT9, KRT13, KRT15, KRT16 and KRT19, and possibly also KRT17, KRT20, and others. Our results, therefore, are consistent with the idea that this disorder is the result of a keratin abnormality. In view of the considerable phenotypic overlap between PC syndromes, it is quite possible that they are all the result of related or allelic keratin gene defects.

Disorders ascribed to defects in keratin genes include EB simplex (KRT5/KRT14),^{13,14} including the Dowling Meara type,¹⁵ epidermolytic hyperkeratosis (KRT1/KRT10),¹⁶⁻¹⁸ and epidermolytic palmoplantar keratoderma (KRT9).^{19,20} In addition, familial keratosis palmaris et plantaris is closely linked to the type I keratin cluster, suggesting the possible in-

volvement of a keratin gene.²¹ Our present linkage results suggest that the hyperkeratosis and EBS-like blistering of PC will also be explained by defective keratin production, structure, organisation, or degradation, but it remains to be established which keratin(s) are involved in pathogenesis. The keratin(s) or regulatory elements involved in the various PCs will be ones which can be active in all the sites involved, namely palmoplantar skin, ventral nail, hair follicles, the corneal epithelium, and possibly sweat glands. Laryngeal mucosa, and, in Jadassohn-Lewandowsky PC, oral mucosa are also involved. Additionally, in Jackson-Lawler PC the premature eruption of teeth suggests an effect of the keratins expressed in fetal gingival and enamel epithelia.²⁶

Adequate data on the keratins expressed by lesional tissue are not available, and no single keratin candidate which fulfils all these criteria is immediately apparent, but because abnormalities are present from the basal layer upwards in Jadassohn-Lewandowsky PC, abnormal keratins 5 or 14 are particular candidates for this variant. The ultrastructural appearances of Jackson-Lawler PC have not been reported. Of the keratin pairs, only those of simple epithelia (K8 and K18) can be excluded for this variant since, exceptionally, both genes are in the chromosome 12q cluster.²² The other type I keratin genes are all possible candidates; genes on chromosome 17q include KRT14, KRT10 (suprabasal expression), KRT16 ("hyperproliferation"), KRT15, and KRT19, and quite possibly KRT17 and KRT20. Many of these genes are also expressed in hair follicles and nail.^{27,28} The nail dystrophy does not begin until a few months of age, which suggests that expression of the defective gene is not constitutive but is a response to mild trauma; this could favour an abnormal induction of K16 expression. Genes encoding the hard keratins of hair and nail, and K12 (wet epithelia) and K13 (cornea) are less likely candidates because of their limited distribution.²² However, the disease state could also result from ectopic keratin gene expression secondary to defects in adjacent regulatory elements. Further work is required to identify the likeliest candidates for defects causing all forms of PC. One useful approach will be definition of the keratin types found in lesional tissues, especially when filaments accumulate in the epidermis^{11,12} or as amyloid.⁸

The demonstration that a keratin defect almost certainly underlies this rare condition has wider implications for understanding epidermal physiology, and also the pathogenesis of commoner skin diseases. The distal subungual hyperkeratosis, probably similar in mechanism to the hyperkeratosis of palmoplantar skin, resembles that seen in some forms of psoriasis, including Reiter's syndrome, and in dermatophyte infection of the nail. Patients with Jackson-Lawler PC have recurrent abscesses resembling the condition hidradenitis suppurativa,⁶ a disabling entity associated with follicular plugging in flexures.²⁹ Familial hidradenitis, and steatocystoma multiplex without nail changes both exist.^{30,31} Investigation of

the defects of keratin in PC is thus relevant to the pathogenesis of these disorders.

Unusual hair is seen in Jackson-Lawler PC; eyebrows are notably bushy and grow perpendicularly out of the skin. This growth may be because of abnormal formation by the hair follicle, but the possibility also exists that the structural hair keratins are themselves abnormal. This hypothesis is supported by reports of abnormally dry, kinked scalp hair,⁴ and by our own observation (Kunkeler and Munro, in preparation) that limb hairs are also abnormally rigid.

Although always likely, our findings provide strong evidence for a keratin gene anomaly underlying an inherited disorder affecting epidermis, nail, hair, and mucosa. In addition, the suggested role for keratins in the phenomenon of natal dentition is intriguing, and may provide useful insights into the regulation of tooth eruption.

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